VERIFICATION OF A TRANSLATION

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1. I am familiar with the English and German languages.

2. I have read the attached German patent application No. 102 44 863.9.

3. The hereto attached English language text is an accurate translation thereof.

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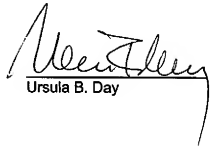

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The attached documents represent a true and accurate copy of the original documents of this patent application.

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Vaccine Against Infections with Oncoviruses such as Feline Leukosis Virus in Cats

Description

The invention relates to a DNA-based vaccine allowing protection of cats against infections with feline leukosis virus.

The feline leukosis virus (FeLV) is a cat-specific virus which is widespread throughout the world and is the trigger of severe diseases and one of the main causes of death in feline populations. Currently, the infection rate is between 12% to 16% in cats both in Europe and in the USA.

Some of the cats can overcome the infection; on the other hand, however, lifelong virus persistence in the organism is possible as well. Latently infected cats are then deemed to be a reservoir of pathogens.

At present, an effective therapy of FeLV infections that would bring about recovery is not possible, the best success being pushing back the disease for some time. Particular types of chemotherapeutic agents can also be applied to cats, but the side effects are highly problematic, as is the case in human medicine. Treatment with interferons is still at an experimental stage.

Virostatic agents are not capable of inactivating the virus and therefore neither lead to a successful cure.

Effective control of FeLV infections is only possible by preventive vaccination.

State of the art

Currently available vaccines are based on either inactivated FeL viruses, proteins produced by recombinant methods, so-called subunit vaccines, or on the use of

genetically modified live vaccines. Apart from insufficient success of vaccination, these types of vaccines, however, show a number of disadvantages.

Thus, preparations of inactivated viruses lead to the desired immunity only in some of the vaccinated animals. These vaccines invariably consist of protein mixtures wherein highly immunogenic antigens have to "compete" with large amounts of other proteins for presentation by the immune system. Furthermore, strong side effects such as allergic reactions and autoimmune diseases may occur after completed vaccination.

A recombinant vaccine consisting of FeLV envelope protein produced using biotechnological methods and added with aluminum hydroxide and saponin is a vaccine frequently used today. Vaccinations using this vaccine provide protection against leukosis in 80% to 95% of the cats (Lutz et al., 1991, J Am Vet Med Assoc 199(10): 1446-52).

However, the risk of fibrosarcomas occurring at the site of vaccination represents a problem. Another drawback of this vaccine is that the immunity being achieved is mainly based on the formation of virus-neutralizing antibodies. However, more recent test results (Flynn et al., 2000, Immunology 101, 120-125) have shown that the cellular immune response is also of great importance in providing protective immunity.

While the use of live vaccines has been found to be effective with regard to the immunity being achieved, such use involves a constant risk in that the virus strains employed develop into new pathogenic virus strains via mutation or recombination. Also, when using such vaccines including all viral structures, it should be noted that differentiation between either infected or vaccinated animals is no longer possible on the basis of the antibody status. For both of these reasons, such vaccines are not suitable in practice.

Another example of a vaccine consisting of infectious or replicable viruses is a recombinant canary poxvirus expressing FeLV surface proteins. In test infections it was possible to protect 83% of the animals from infection (Jarrett et al., 1993, J of Virology: 2370-2375). This vaccine, however, shows the disadvantages of a live vaccine with regard to unpredictable recombinations and, in addition, production and characterization thereof are relatively complex and thus expensive.

Apart from such traditional and modern recombinant vaccines, there is the option of vaccinating using DNA expression constructs, wherein it is only the information relating to particular immunogenic portions of the pathogen in the form of DNA that is administered to the vaccinee. Following vaccination, the FeLV antigens are expressed by the cells of the vaccinated cat, thereby stimulating an immune response to the virus.

The option of achieving an immune response to an antigen by injecting DNA expression constructs encoding this antigen has first been published by Tang and Ulmer for mice (Tang et al., 1992, Nature 365, 152-154; Ulmer et al., 1993 Science 259, 1745-1749) and has been demonstrated in a large number of species since then. It can be assumed that the general principle of vaccination with nucleic acids encoding immunogens is applicable to all higher animals. Regarding the selection of suitable antigens, their code in nucleic acid sequences, and the selection of a suitable vaccination regime, however, each use poses a number of problems to a person skilled in the art, some of which being difficult to overcome, resulting in the fact that no DNA-based vaccine has been included in clinical phase 2 or 3 testing as yet.

The vaccination of cats with expression constructs to express the "env" and "gag" genes has been described in the French patent document FR 2 751 223. However, the disclosure of the invention outlined therein is insufficient, i.e., no expression or immunization tests or results thereof are shown. Apparently, this is a speculative application not being based on inventive activity.

Experiments relating to DNA immunization in the field of FeLV are well-known (Jarrett et al., 2000 Immunology 101, 120-125) but have failed to provide convincing success. In the paper cited above, the total genome having a polymerase deletion has been inoculated as expression construct. However, the clinical success of vaccination has not been reflected in protection of cats from infection or viremia. Apart from this practical disadvantage of the cited vaccination test, the use of deletion mutants or genomes thereof in vaccination is disadvantageous in view of the risk that new infectious pathogens are formed from a deleted virus via recombination with endogenous or exogenous viral sequences.

In contrast to the cited paper by Jarrett, the efforts of the present invention were aimed to express isolated FeLV antigens only. However, preliminary experiments showed that inoculation of expression constructs encoding homologous wild-type sequences of the "env" and "gag" genes of FeLV under control of the cytomegalovirus (CMV) early-immediate promoter failed to induce antibody production in cats. Likewise, further experiments showed that the respective sequences were not expressed - or only to a very small degree - in human cell lines and those obtained from cats. This phenomenon is well-known in sequences of HI virus and other lentiviruses (Wagner et al., 2000, Hum Gene Ther 11(17), 2403-2413). The expression of wild-type sequences in an infected cell depends on the previous expression of the virus-encoded "rev" protein.

The expression control of the FeLV retrovirus, which does not belong to the class of lentiviruses, is unknown, and a mechanism similar to "rev" control has neither been demonstrated nor postulated in the literature.

Also, codon usage optimization within the expression construct to codons preferentially used in mammals is known to enable a substantial increase in protein expression (Grantham et al., Nucleic Acids Res 1980, 9:1893-912). This method has been employed successfully to raise the expression level of various viral structural proteins of HIV-1 and SIV. The effect relies on circumventing the "rev"-dependent transport paths for the extremely AT-rich transcript of these late proteins in the rep-

lication cycle of lentiviruses. Thus, using codon optimization of the DNA sequences of the "env" and "gag" proteins of human HI virus, it has been possible to achieve much higher antibody titers against these synthetic antigens in mice compared to the use of wild-type sequences (Haas et al., 1998, J Virol. 72: 1497-503; Wagner et al., Hum Gene Ther. 2000, 17: 2403-13). Preparation and use in vaccination against HIV-1 of sequences optimized in this way are also known from WO 00/029561 and WO 97/48370.

Another problem lies in the use of the DNA encoding the immunogenic antigens or parts thereof. A disadvantage of the vectors currently used for DNA transport (transfection) is that either vectors of viral origin, which cause problems concerning the aspect of safety (Lehrman, 1999, Nature 401: 517-518), or plasmids are used. Plasmids are produced by bacterial fermentation and therefore, in addition to the desired gene, also contain DNA necessary for their propagation and selection, and normally resistance genes against antibiotics used in bacterial fermentation. These problems are discussed in detail in WO 98/21322. It should be noted that the use of gene expression constructs on the basis of plasmid DNA involves the inherent risk of disseminating antibiotic resistance genes, which is especially irresponsible in the context of large-scale vaccination campaigns.

Covalently closed minimalistic DNA constructs such as those disclosed in EP 0 914 318 B1 represent another type of DNA vectors. Especially their use in the form of peptide-coupled DNA constructs results in a surprising, qualitatively improved immune response compared to unmodified DNA (see also DE 101 56 679.4 and DE 101 56 678.6).

Apart from the disadvantages associated with previous gene transfer methods, no success in developing an effective and safe vaccine against FeLV has been achieved as yet. Up to now, the treatment of FeLV infection is restricted to boosting the body defenses of the animals and treating accompanying and secondary infections. Available vaccines involve the side effects mentioned earlier.

Given this state of research, the object of the present invention is to provide a vaccine which results in cellular and humoral immunity against selected antigens of FeLV in cats and protection from infections with FeLV and takes into account the aspect of safety. It is also intended to do without conventional adjuvants so that the risk of fibrosarcoma formation at the site of injection can be ruled out. Another object of the present invention is to provide expression constructs resulting in expression of FeLV antigens in mammal cells in measurable quantities.

Inventive solution and advantages of the invention

According to the invention, said object is accomplished by immunizing cats with a mixture (cocktail) of synthetically produced codon- and splicing signal-optimized DNA sequences encoding the structural proteins ("gag") and the most important FeLV membrane protein ("env").

The "gag" gene sequence codes for the viral structural proteins of the inner viral packaging and the "env" gene sequence for the envelope proteins. The protein possessing the highest immunogenicity of those proteins encoded in the "env" sequence is the gp70 glycoprotein. Virus-neutralizing antibodies against gp70 are produced in the cat organism. These antibodies represent the first immune response after invasion of the pathogen into the body, which alone might possibly be sufficient to overcome the infection.

There is an ongoing controversial discussion whether membranous proteins or secretory proteins are better suited to induce virus-neutralizing antibodies. For this reason, two different constructs encoding "env" were produced. The p15 sequence of the "env" gene sequence is known to contain at least one sequence section having an immunomodulatory effect (Haraguchi et al., 1997, Journal of Leukocyte Biology, 61, 654-666), which suppresses antibody formation so that, in addition to a construct encoding gp70 and p15 (gp85), another one was produced which contains gp70 only and thus results in expression of the secretory "env" protein without transmembrane portion.

A vaccine that can induce both virus-neutralizing antibodies against gp70 and a T cell-mediated immune response therefore constitutes a significant improvement over vaccines available so far and could optionally be employed in therapy of infected cats as well.

To express more antigen *in vivo* and trigger a stronger immune response that becomes apparent in effective and long-lasting protection against FeLV infection, the wild-type sequences of "gag" and "env" were optimized. Optimization is understood to be codon adaptation, also referred to as codon usage optimization.

Any amino acid can be encoded by several codons. The frequency of reading the single codons during translation strongly varies between viruses, bacteria and vertebrates so that the abundance of the respective tRNAs in a cell varies correspondingly. The codon usage frequency of viral genomes partially differs from that of a host cell, this probably being an element of expression control of viruses. By adapting the sequence to the host-specific codon usage pattern, the viral control mechanisms can be evaded and the expression of antigen substantially increased.

The experiments were therefore aimed at achieving much higher antigen expression levels by transcribing the viral sequences into sequences representing optimum codon usage for vertebrate genomes. To this end, a cloning strategy was developed, allowing synthesis of the optimized DNA sequence from oligonucleotides.

The synthetic sequences were inserted in plasmids, multiplied in *E. coli*, sequenced for control, and subsequently transfected in cells of a cat cell line in order to test the expression of the encoded proteins.

Expression of the antigens was detected using Western blots.

Expression of the proteins by the FeLV wild-type sequences (wt) of "env" and "gag" was detected by Western blotting. Immunoprecipitation allowed detection of both "env" and "gag" only in the form of very weak bands. Surprisingly, this was also the

case with codon-optimized "env" sequences. It was only with "gag" where codon optimization gave significantly improved expression, as demonstrated in Fig. 1. After checking a number of other hypotheses intended to explain the poor expression of the synthetic genes, the synthetic "env" sequence was examined for splicing signals (splice sites) predicted by bioinformatic methods. A number of splicing signals predicted by the program used were deleted by point mutation so as to verify the hypothesis that the appearance of such structures would impede gene expression in association with the promoter being used. Surprisingly, as a result of this measure, it was possible to detect "env" as a strong protein band in the Western blot (see Fig. 2). The synthetic antigen sequences according to the invention give successful and enhanced antigen expression, as seen in the strong bands.

A correlation between the expression level of an expression system and the resulting level of immune response has been assumed in general, although numerous findings suggest that there is no linear correlation and that treatment with expression vectors would not necessarily result in immunity at the desired level in each case (Wherry et al., Journal of Immunology 2002, 168, pp. 4455-4461). After obtaining *in vitro* expression results, mice were therefore immunized with peptide-linked expression vectors encoding the codon-optimized and splicing signal-optimized "env". The advantages of such peptide-linked constructs in inducing immune response have been explained in detail in the documents EP 0 941 318 B and DE 101 56 678 A. To elucidate the immunological significance of the p 15 protein of "env" in triggering an immune response, both inventive sequences encoding "env" were used (SEQ ID NO: 7, 8 as well as 9 and 10). The sera of immunized mice were examined for specific antibodies against the FeLV virus protein "env" using Western blotting. The antibody levels following secondary immunization in week 4 clearly demonstrate that the synthetic constructs result in strong stimulation of antibody formation *in vivo* as well. In comparison, five out of six mice in group 4 showed strong immune response to the inventive antigen sequence, whereas the wild-type (group 1) induced weak immune response in only two out of six animals (see Fig. 3).

Plasmids can be used as DNA expression constructs, although, according to the invention, the use of minimalistic immunologically defined expression constructs is preferred. These constructs are linear, covalently closed expression cassettes consisting only of a CMV promoter, an intron, the respective gene sequence, and a polyadenylation sequence. These covalently closed, minimalistic DNA constructs will be referred to as MIDGE vectors hereinbelow (MIDGE: minimalistic immunologically defined gene expression vector); see EP 0 941 318 B1. The MIDGE constructs are advantageous in that structures not being essential to the medical effect thereof can be dispensed with, thereby avoiding the drawbacks of conventional gene ferries.

For transfection, biological, chemical and/or physical prior art methods can be employed, such as transfection via ballistic transfer. In a preferred embodiment of the invention, transfection is achieved by means of intradermal injection using syringes or needleless injection devices.

Further advantageous methods are biological transfection methods such as peptide-mediated gene transfer wherein, for example, a DNA expression construct encoding at least the inventive "env" and at least the inventive "gag" sequence of FeLV is covalently bound to an oligopeptide, preferably the nuclear localization sequence (nuclear localization signal: NLS) from SV-40 simian virus.

In view of the positive results in the mouse experiment, cats were immunized with the expression constructs and examined for their antibody status.

Further advantageous embodiments of the invention will be apparent from the subclaims and the description. The surprising effect of the medication according to the invention (as a vaccine in FeLV therapy) and the method of the invention will be apparent from the figures and examples wherein:

MIDGE-NLS-FeLVenvgp85(-splice)	NLS-coupled MIDGE vector encoding the codon- and splicing signal-optimized "env" sequence with p15 (gp85)
MIDGE-NLS-FeLVenvgp70(-splice)	NLS-coupled MIDGE vector encoding the codon- and splicing signal-optimized "env" sequence without p15 (gp70)
wt MIDGE-NLS	NLS-coupled MIDGE vector encoding the wild-type "env" gene
mAb against gp70	monoclonal antibody against gp70
Positive control	Leucogen vaccine
Buffer	PBS, negative control

The attached sequence listing illustrates the following sequences:

SEQ ID NO:	Name of sequence/description
1	DNA sequence of the wild-type "env" gene
2	DNA sequence of the wild-type "gag" gene
3	protein sequence of the wild-type "env" gene
4	protein sequence of the wild-type "gag" gene
5	DNA sequence of the codon-optimized "gag" gene
6	protein sequence of the codon-optimized "gag" gene
7	DNA sequence of the codon- and splicing signal-optimized "env" gene (gp85)
8	DNA sequence of the codon- and splicing signal-optimized "env" gene (gp70)
9	protein sequence of the codon- and splicing signal-optimized "env" gene (gp85)
10	protein sequence of the codon- and splicing signal-optimized "env" gene (gp70)

Fig. 1 shows a comparison of *in vitro* expression of the wild-type "gag" protein and codon-optimized "gag" protein. Lysates of cat cells previously transfected with the following constructs were applied:

Lane 1+2: expression vectors encoding wt "gag"
Lane 3: expression vectors encoding codon-optimized "gag"
Lane 4: non-infected cat cells, negative control
Lane 6: virus-infected cat cells, positive control
Lane 7: Boa protein marker

Expression by the wild-type results in very weak protein bands (1 and 2), whereas strong expression is achieved by the sequence according to the invention (3). Infected cat cells were used for comparison (6).

Fig. 2 shows a comparison of *in vitro* expression of the wild-type "env" gene and codon- and splicing signal-optimized "env" sequence (gp85). Lysates of cat cells previously transfected with the following constructs were applied:

Lane 1: Boa protein marker
Lane 2: non-infected cat cells, negative control
Lane 3: virus-infected cat cells, positive control
Lane 5: virus-infected cat cells, positive control
Lane 6: non-infected cat cells, negative control
Lane 7: additional negative control for specific "env" detection
Lane 9: expression vectors encoding the codon- and splicing signal-optimized FeLVenvgp85(-splice)
Lane13: expression vectors encoding the codon-optimized FeLVenvgp70(+splice)
Lane 15: expression vectors coding for wild-type "env"

The wild-type gives no visible protein band (15). In contrast, the codon-optimized sequence (13) gives a weak band, whereas the codon- and splicing signal-optimized sequence of the "env" protein (9) gives a strong protein band.

Fig. 3 shows *in vivo* results after immunization of mice with expression constructs encoding "env". Antibody determination was performed in week 4 following secondary immunization. In group 3 (immunization with inventive gp85 sequence), 3 out of 6 mouse sera are antibody-positive, in group 4 (immunization with inventive gp70 sequence), 5 sera are strongly positive and 1 is weakly positive. In contrast, the wt-immunized animals in group 5 show very weak positive signals in only two cases. The experiment demonstrates that the optimized sequences, compared to the wild-type sequences, give markedly enhanced antibody formation *in vivo* as well, thus confirming the results of the *in vitro* experiments.

Example 1: Wild-type (wt) sequences

The wild-type sequences of the selected antigens were obtained from the blood of infected cats. The DNA sequence for the wt "env" is represented in SEQ ID NO: 1, for the wt "gag" in SEQ ID NO: 2, and the corresponding amino acid sequences in SEQ ID NO: 3 ("env") and SEQ ID NO: 4 ("gag").

Primers for wt "gag" :
In order to remove two Eco31I restriction sites, 3 PCRs were performed using the following mutation primers:
gag-mut1-rneu:
AATTAAGAGCTCCACGTCTCCCCCCGCTAACAGCAACTGGCG
gag-mut2-l:
AATTAAGAGCTCCAGGTCTCCGGGGCTCCGCGGGGCTGCAAGACG
gag-mut3-r:

AATTAAGAGCTCCACGTCTCCTTCCCTTTTGTTGTATATCTTTTCTGC
gag-mut4-l:
AATTAAGAGCTCCAGGTCTCCGGAAACCCCAGAGGAAAGGGAAGAAAG

After ligating the three sequences obtained, a PCR was performed using the primers:
FeLVgag-l:
CGGATAAGGTACCATGGGCCAAACTATAACTACC
FeLVgag-r:
TTCTCAGAGCTCTTAGAGGAGAGTGGAGTTGGCGGG

Primers for wt "env":
envl: CGGATAAGGTACCATGGCCAATCCTAGTCCACC
envr: AGTTCTCAGAGCTCTTAGGCTGTTTCAAGGAGGGCTT

Example 2: Codon Optimization

The codon usage table for cats was selected from the Codon Usage Database (http://www.kazusa.or.ip/codon/). For each amino acid of the two wild-type sequences, the codon most frequently coding for this amino acid was used. The following restrictions were applied:
If an amino acid appeared more than three times in a row, the second most frequent codon was used from the fourth amino acid on. In this way, sudden extreme decrease of tRNA should be avoided and effectiveness of transcription ensured.

Sequences including C and immediately following G were avoided so as to rule out uncontrolled immunostimulatory effects.

In order to avoid Eco31I, KpnI and SacI restriction sites, all sequences having the base sequences GAGCTC, GGTACC, GGTCTC and GAGACC were removed by selecting alternative codons likewise frequently used.

Example 3: Cloning of FeLVenv

Oligonucleotides having a length between 18 and 28 bases were purchased (Tib-Molbiol). In total, 51 oligonucleotides were joined by annealing and ligating. The overhang was 4 bases. Care was taken that overhangs occurred only once and were non-palindromic. Each single oligonucleotide was hybridized with strand and counter-strand by adding kinase buffer to the two single oligonucleotides (strand and counter-strand), heating to about 80°C, followed by slow cooling to room temperature. Subsequently, ATP and polynucleotide kinase (PNK) were added and the oligonucleotides were phosphorylated for one hour. Thereafter, in a first step, oligonucleotides neighboring each other were combined and ligated (oligonucleotides 1+2, oligonucleotides 3+4). After ligating for 1 h, an aliquot from the ligation batch of the oligonucleotides 1+2 and an aliquot from the ligation batch 3+4 were combined. An aliquot of the last ligation batch was used to perform a PCR with flanking primers. If a PCR product having the proper expected length had formed, it was intermediately cloned into the TOPO vector pCR 2.1 and the sequence was verified. The other fragments of the complete gene were treated in an analogous fashion. Four fragments were obtained. The individual fragments were cut from the intermediately cloned plasmid using EcoRI and ligated with ligase after digestion with Eco31I. The complete ligation product of proper length was digested with BamHI and SacI and cloned into the similarly cut vector pMCV1.4 after gel extraction. Subsequently, the sequence was verified by sequencing. The resulting plasmid was named pMCV1.4-FeLVenv.

Primers for these 4 synthesized fragments:

Fragment 1:
Left primer: ATATTGGATCCCATGGCCAACCCCTCCC
Right primer: ATTATGGTCTCCTGCTGCTTCTTCCTGTCTGTGG
Fragment 2:
Left primer: TAATAGGTCTCCAGCAGCAGACCTACCCCT
Right primer: TAATAGGTCTCTGTGAACAGGGCAATGGGGTCA

Fragment 3:
Left primer: TATTTGGTCTCTTCACAGTGTCCAGGCAGGTGTC
Right primer: TATTAGGTCTCAGCTTGTGCTGGGGGGTGG
Fragment 4:
Left primer: AATAAGGTCTCCAAGCTGACCATCTCTGAGGTGT
Right primer: ATTAAGAGCTCTCAGGCTGTTTCCAGC
Complete sequence:
Left primer: ATATTGGATCCCATGGCCAACCCCTCCC
Right primer: ATTAAGAGCTCTCAGGCTGTTTCCAGC

Example 4: Cloning strategy for LeadFeLVenv

For successful processing of the "env" protein, a signal sequence (leader sequence) was cloned in front of the codon-optimized "env" sequence. This signal sequence was produced from a total of 8 ODNs with a length of between 22 to 30 bp by annealing and ligating. From the last ligation step, a PCR was performed to amplify the leader sequence.

Primers for the complete signal sequence:
Left Primer: ATTGCCGGTACCATGGAGTCCCCCACCCACC
Right Primer: ATCAGAGGTCTCCCATGCCAATGTCAATGGTGAAC

An Eco31I recognition sequence was generated at the 3' end of the PCR product, which, following digestion, creates an overhang having reverse complementarity to the overhang of the following PCR product produced at the 5' end after similar digestion.

PCR for FeLVenv:
This generates an Eco31I recognition sequence at the 5' end of the sequence.

Primers used:
Left primer: GATCTGGGTCTCCATGGCCAACCCCTC

Right primer: ATTAAGAGCTCTCAGGCTGTTTCCAGC

Following digestion of the two PCR products with Eco31I, they were purified and ligated together. The ligation product was employed in a PCR wherein a recognition sequence for KpnI was generated at the 5' end and for SacI at the 3' end.

Primers used:
Left primer: ATTGCCGGTACCATGGAGTCCCCCACCCACC
Right primer: ATTAAGAGCTCTCAGGCTGTTTCCAGC

The PCR product was digested with KpnI and SacI and cloned into the vector pMCV1.4 cut in the same way. The resulting plasmid was named pMCV1.4-LeadFeLVenv.

Example 5: Cloning strategy for LeadFeLVenvgp85

The complete "env" polyprotein consisting of gp70 and p15 was cloned. To this end, the wild-type p15 sequence was amplified by PCR from the plasmid pMCV1.4-FeLVenvp15 and cloned behind the pMCV1.4-LeadFeLVenv mentioned above.

An Eco31I recognition sequence was generated at the 5' end during the amplification of p15.

1st PCR:
Primers used:
Left primer: AATTATGGTCTCGCAGTTCAGACAACTACAAATGGC
Right primer: AATTATGAGCTCTCAGGGCCTGTCAGGGTC

2nd PCR:
The sequence of LeadFeLVenv was amplified to generate a recognition sequence at the 3' end.

Primers used:
Left primer: AATTATGGTACCATGGAGTCCCCCACCC
Right primer: TATAATGGTCTCAACTGGGCTGTTTCCAGCAGGGC

Following digestion of the two PCR products with Eco31I, they were ligated together. The ligation product was employed in a PCR using the following primers:
left primer: AATTATGGTACCATGGAGTCCCCCACCC
right primer: AATTATGAGCTCTCAGGGCCTGTCAGGGTC

In this way, a KpnI recognition sequence was generated at the 5' end and a SacI recognition sequence at the 3' end. Following digestion of the PCR product with KpnI and SacI, the product was ligated and cloned into pMCV1.4 cut in the same way. The resulting plasmid was named pMCV1.4-LeadFeLVenvgp85.

Example 6: Splicing signal optimization of LeadFeLVenvgp85(-splice):

The DNA sequence of LeadFeLVenv was examined for possible splicing signal sequences (splice sites) under http://www.fruitfly.org/seq tools/splice.html. A splice site was recognized with 97% probability between the bases 100 and 140. After substituting base 119 (A with G, amino acid substitution of Gln with Arg), no potential splice site was recognized any longer (lower limit = 40% probability). The mutated sequence was generated and cloned as follows:

PCR for the generation of the mutated sequence:
First of all, a fragment (1) consisting of the bases 1-123 of LeadsynFeLVenv was amplified using PCR. The forward primer employed generates the recognition sequence of the KpnI restriction enzyme at the 5' end of the PCR product.

The sequence of the reverse primer was selected in such a way that the PCR product has the mutation (base 119 = G). In addition, the PCR generates the recognition sequence of the restriction enzyme Eco31I-site at the 3'-end of the PCR

product. Basically, Eco31I generates a 4-base 5' overhang of the bases 2-5 downstream of the recognition sequence.

The 4-base 5' overhang generated by digesting the PCR product with Eco31I at the 3' end of fragment 1 corresponds to the bases 120-123 of the LeadFeLVenv sequence. This sequence in turn corresponds to the overhang that is also generated when digesting LeadFeLVenv with the BgLII restriction enzyme because the bases 119-124 of LeadFeLVenv represent the recognition sequence of BgLII.

Thus, the bases 1-123 are first cut out of the pMCV1.4-LeadFeLVenv construct by means of KpnI and BgLII.

Following digestion of the fragment 1 PCR product (including mutation of base 119 = G) with KpnI and Eco31I, the product can be ligated and cloned into the KpnI- and BgLII-cut and purified vector pMCV1.4LeadFeLVenv. The resulting plasmid was named pMCV1.4-FeLVenvgp70(-splice).

Primers used:
Left primer: 5' ATTGCCGGTACCATGGAGTCCCCCACCCACC
Right primer: 5' ATATTAGGTCTCAGATCCGGGGGGGGGAGGG

In an analogous manner, the fragment 1 PCR product was ligated and cloned into the KpnI- and BgLII-cut and purified vector pMCV1.4-LeadFeLVenvgp85(-splice). The resulting plasmid was named pMCV1.4-FeLVenvgp85(-splice).

Example 7: Cloning strategy for FeLVgag

Cloning of FeLVgag was performed corresponding to the procedure described under 3. The sequence was produced via oligonucleotides initially annealed and ligated into three single fragments. The sequence was constructed from a total of

2 × 31 oligonucleotides (forward and reverse strands). The fragments were used as templates in a PCR and amplified using the following primers:

Fragment 1:
Left Primer: ATATTGGTCTCAGGAGAGGGACAAGAAGAG
Right Primer: AATATGGTCTCTCAGCCTGCTGGCGATGGGGC
Fragment 2:
Left primer: ATTATGGTCTCTGCACCTGAGGCTGTACAGGC
Right primer: AATATGGTCTCGGTGCTCCCTGCCGGCGGGGGTGCA
Fragment 3:
Left primer: ATTATGGTCTCTGCACCTGAGGCTGTACAGGC
Right primer: AATATGGTCTCTCTCCTCCTGCCTCTGC
Primers for the complete fragment:
Left primer: ATATTGGTCTCAGGAGAGGGACAAGAAGAG
Right primer: AATATGGTCTCTCTCCTCCTGCCTCTGC

Fragments 1, 2 and 3 were intermediately cloned into the TOPO vector pCR2.1, subsequently digested with Eco31I and extracted. The ligation product from 1, 2 and 3 was digested with KpnI and SacI and cloned into pCMV1.4. The resulting plasmid was named pCMV1.4FeLVgag.

Example 7: Transfection of cells, detection of expression

Feline cells of the f3201 cell line were transfected with the plasmids pMCV1.4-FeLVenvgp85(-splice), pMCV1.4-FeLVenvgp70(-splice), FeLVgag and the wt-containing plasmids pMOK for "env" and "gag" using electroporation at 250 V and 1050 µF.

Detection of the expressed proteins was performed using the Western blot method. Monoclonal mouse antibodies were used in detection. Positive control: FeLVA-infected cells of the f3201 cell line.

Example 8: Production of peptide-linked MIDGE

The plasmids pMCV1.4-FeLVenvgp85(-splice), pMCV1.4-FeLVenvgp70(-splice) and pMCV1.4-FeLVgag were completely digested with the Eco31I restriction enzyme at 37°C overnight. Two DNA fragments were generated as a result of restriction digestion. One fragment comprised the kanamycin resistance gene and other sequences necessary for propagation of the plasmid in bacteria. The other fragment comprised the sequences intended to be part of the MIDGE DNA: enhanced CMV promoter, chimeric intron, corresponding gene sequence and polyadenylation sequence from SV-40. Using the T4 DNA ligase enzyme, the 5'-phosphorylated hairpin oligonucleotides (TIBMolBiol, Berlin) 5'-PH-GGGAGTCCAGT xT TTCTGGAC-3' and 5'-PH-AGG GGT CCA GTT TTC TGG AC-3' were ligated to the MIDGE-forming DNA fragment in the presence of the Eco31I restriction enzyme at 37°C overnight. The reaction was quenched by heating to 70°C. The resulting mixture of nucleic acids was treated with the T7 DNA polymerase enzyme. The MIDGE DNA was purified by means of anion exchange chromatography and precipitated with isopropanol (see EP 0 941 318 B1).

Production of the peptide-linked ODNs:

The NLS peptide PKKKRKV was linked to the ODNs in two steps. Initially, the modified oligonucleotide 5'-PH-d(GGGAGTCCAGT xT TTCTGGAC, wherein xT is an amino-modified thymine base with a C_2 amino linker) (0.1 mM) was activated with sulfo-KMUS (5 mM) in PBS at room temperature (RT). The reaction was quenched after 120 min by adding 50 mM tris(hydroxymethyl)aminomethane and the activated ODN was obtained after ethanol precipitation (300 mM NaOAc, pH 5.2, 5.5 mM $MgCl_2$, 70% ethanol), centrifugation and a single wash with 70% ethanol. The ODN (0.1 mM) thus obtained was dissolved in PBS and reacted with the peptide (0.2 mM) for one hour at room temperature. The reaction was monitored using gel electrophoresis (3%) and ethidium bromide staining. The resulting NLS-linked ODN was purified by means of HPLC and used in the synthesis of the MIDGE-NLS constructs as described above.

Example 9: Antibody assay in mice

Five vaccination groups were formed, each one including six BALB/c mice. The basic antigens in all groups (except the control groups) were the optimized sequences of the "env" protein with and without the immunomodulatory protein p15. The coding sequence and the cytomegalovirus promoter (CMV) placed in front of the sequence are employed as linear double-stranded molecules according to Example 8. PBS buffer, conventional vaccine (Leucogen) and the wild-type "env" protein were used as controls. Initial immunization (50 µg of DNA, 1 mg/ml, i.d.) was followed by a secondary immunization (boost) on day 15. Blood was collected on days 14, 28 and 42. The blood samples were assayed for specific antibodies against "env".

Group	Mice	Antigen used	Purpose
1	6	Leucogen	Positive control
2	6	PBS buffer	Negative control
3	6	FeLVenvgp85(-splice)	Antibody detection
4	6	FeLVenvgp70(-splice)	Antibody detection
5	6	Wild-type "env"	Positive control

Example 10: Immunization of cats

To test the capability of the synthetic sequences of inducing a humoral and cellular immune response in cats, the following vaccination regime was formulated:

Table 1: Set-up of vaccination groups

Group	Cats	Antigen used	Purpose
1	5	FeLVenvgp85(-splice) FeLVgag	Determine antibody and cytokine status
2	5	FeLVenvgp70(-splice) FeLVgag	Determine antibody and cytokine status, Compare with group 1
3	2	PBS buffer	Negative control
4	3	Leucogen	Positive control

Each cat of the first two groups is immunized twice with a total of 600 µg of DNA dissolved in PBS buffer. The peptide-linked expression constructs are administered by intradermal injection into the neck. The immune response is monitored for 12 weeks. Secondary immunization is performed in week 4. Determining the cytokine status from blood samples collected on a weekly basis is intended to provide conclusions as to the direction of the immune response (Th1, Th2). IL-4 was determined as indicator of a TH2 response and IL-2 and interferon-gamma as indicators of a predominantly TH1-directed immune response. The Leucogen vaccine contains recombinant "env" protein and is used as positive control.

Antibodies against the antigens used were determined using Western blotting and ELISA methods.

The mRNA amount of the cytokines IL-2, IL-4 and interferon-gamma was determined using real-time PCR.

Claims:

1. A DNA expression construct for the expression of gene products of feline leukosis virus (FeLV) in cat cells, constituted of a promoter sequence operable in felids and at least one nucleotide sequence encoding an FeLV structural protein and/or membrane protein, wherein the coding sequence of the FeLV is codon-optimized and has neither normal nor cryptic splice donor and/or acceptor sequences.

2. The DNA expression construct according to claim 1, said expression construct including the codon-optimized nucleotide sequence (SEQ ID NO: 5) encoding the gag structural protein.

3. The DNA expression construct according to claim 1, said expression construct including the codon- and splicing signal-optimized nucleotide sequence (SEQ ID NO: 8) encoding the env-gp70 membrane protein.

4. The DNA expression construct according to claim 1, said expression construct including the codon- and splicing signal-optimized nucleotide sequence encoding the env-gp70 membrane protein, which sequence is extended by the nucleotide sequence encoding the immunogenic protein p15 (SEQ ID NO: 7).

5. The DNA expression construct according to one or more of the preceding claims, said structural and/or membrane proteins being entirely or partially encoded by the corresponding nucleotide sequences.

6. The DNA expression construct according to at least one of claims 1 to 5, said expression construct being a plasmid.

7. The DNA expression construct according to at least one of claims 1 to 5, wherein the immunizing polynucleotide sequences are present as expression constructs consisting of covalently closed linear deoxyribonucleic acid molecules having a linear double-stranded region, and wherein the double strand-forming single strands are linked via short single-stranded loops of deoxyribonucleic acid nucleotides, said double strand-forming single strands consisting only of the coding sequence under control of a promoter operable in the animal to be vaccinated and a terminator sequence.

8. The DNA expression construct according to at least one of the preceding claims, said expression construct being covalently linked to one or more peptides.

9. The DNA expression construct according claim 8, said peptide consisting of 3 to 30 amino acids, at least half of which being basic amino acids from the group of arginine and lysine.

10. The DNA expression construct according to claim 9, said peptide having the amino acid sequence PKKKRKV (proline-lysine-lysine-lysine-arginine-lysine-valine).

11. A medication, especially a vaccine, for generating preventive and/or therapeutic immunity in felids, especially in cats, including the DNA expression construct according to claims 1 to 10.

Abstract:

The invention relates to a vaccine which can induce protection against diseases as a result of lentivirus infection, particularly infection with feline leukosis virus (FeLV). Such a vaccine includes codon- and splicing signal-optimized DNA sequences encoding structural proteins and the most important membrane protein of FeLV.

SEQ ID NO: 1 DNA sequence of wild-type "env"

ATGGCCAATCCTAGTCCACCCCAAATATATAATGTAACTTGGGTAATAACCAATGTACAA
ACTAACACCCAAGCTAACGCCACCTCTATGTTAGGAACCTTAACCGATGCCTACCCTACC
CTACATGTTGACTTATGTGACCTAGTGGGAGACACCTGGGAACCTATAGTCCTAAACCCA
ACCAATGTAAAACACGGGGCACGTTACTCCTCCTCAAAATATGGATGTAAAACTACAGAT
AGAAAAAAACAGCAACAGACATACCCCTTTTACGTCTGCCCCGGACATGCCCCGTCGTTG
GGGCCAAAGGGAACACATTGTGGAGGGGCACAAGATGGGTTTTGTGCCGCATGGGGATGT
GAGACCACCGGAGAAGCTTGGTGGAAGCCCACCTCCTCATGGGACTATATCACAGTAAAA
AGAGGGAGTAGTCAGGACAATAGCTGTGAGGGAAAATGCAACCCCCTGGTTTTGCAGTTC
ACCCAGAAGGAAGACAAGCCTCTTGGGACGGACCTAAGATGTGGGGATTGCGACTATAC
CGTACAGGATATGACCCTATCGCTTTATTCACGGTGTCCCGGCAGGTATCAACCATTACG
CCGCCTCAGGCAATGGGACCAAACCTAGTCTTACCTGATCAAAAACCCCATCCCGACAA
TCTCAAACAGGGTCCAAAGTGGCGACCCAGAGGCCCCAAACGAATGAAAGCGCCCCAAGG
TCTGTTGCCCCCACCACCATGGGTCCCAAACGGATTGGGACCGGAGATAGGTTAATAAAT
TTAGTACAAGGGACATACCTAGCCTTAAATGCCACCGACCCCAACAAAACTAAAGACTGT
TGGCTCTGCCTGGTTTCTCGACCACCCTATTACGAAGGGATTGCAATCTTAGGTAACTAC
AGCAACCAAACAAACCCCCCCCCATCCTGCCTATCTACTCCGCAACACAAACTAACTATA
TCTGAAGTATCAGGGCAAGGAATGTGCATAGGGACTGTTCCTAAAACCCACCAGGCTTTG
TGCAATAAGACACAACAGGGACATACAGGGGCGCACTATCTAGCCGCCCCCAACGGCACC
TATTGGGCCTGTAACACTGGACTCACCCCATGCATTTCCATGGCGGTGCTCAATTGGACC
TCTGATTTTTGTGTCTTAATCGAATTATGGCCCAGAGTGACTTACCATCAACCCGAATAT
GTGTACACACATTTTGCCAAAGCTGTCAGGTTCCGAAGAGAACCAATATCACTAACGGTT
GCCCTTATGTTGGGAGGACTTACTGTAGGGGGCATAGCCGCGGGGGTCGGAACAGGGACT
AAAGCCCTCCTTGAAACAGCCTAA

SEQ ID NO: 2 DNA sequence of wild-type "gag"

ATGGGCCAAACTATAACTACCCCCTTGAGCCTCACCCTCAACCACTGGTCTGAGGTTCAG
GCACGGGCCCGTAATCAGGGTGTCGAAGTCCGGAAAAAGAAATGGATTACACTGTGTGAA
GCCGAATGGGTAATGATGAATGTAGGTTGGCCCCGAGAAGGAACTTTCACCATTGACAAT
ATTTCACAGGTCGAGGAGAGAATCTTCGCCCCGGGGCCATATGGACACCCAGATCAAATC
CCTTATATTACCACGTGGAGATCCCTAGCCACAGACCCCCCTCCATGGGTTCGCCCATTC
CTACCCCCTCCTAAGCATCCCAGGACAGATCCTCCCGAGCCTCTTTCGCCGCAACCTCTT
GCGCCGCAACCCTCTTCCCCCCACCCCGTCCTCTACCCCGTTCTCCCCAAACCAGACCCC
CCGAAGGCGCCTGTATTACCACCCAATCCTTCTTCCCCTTTAATTGATCTCTTAACAGAA
GAGCCACCTCCCTATCCTGGGGGTCACGGGCCAACACCGCCGTCAGGCCCTAGAACCCCA
ACTGCCTCCCCGATTGCCATCCGGCTGCGAGAACGACGAGAAAATCCAGCTGAGAAATCT
CAAGCCCTCCCCTTAAGGGAAGACCCAAACAACAGACCCCAGTACTGGCCATTCTCGGCC
TCTGACCTGTACAATTGGAAATTGCATAACCCCCCTTTCTCCCAGGACCCAGTGGCCCTA
ACTAACCTAATTGAGTCCATTTTAGTGACACATCAGCCAACCTGGGACGACTGCCAACAG
CTCTTACAGGCTCTCCTGACGGCAGAGGAGAGACAAAGGGTCCTCCTTGAAGCCCGAAAG
CAAGTTCCAGGCGAGGACGGACGGCCAACCCAGCTGCCCAATGTCGTTGACGAGGCTTTC
CCCTTGACCCGTCCCAACTGGGATTTTTGTACGCCGGCAGGTAGGGAGCACCTACGCCTT
TATCGCCAGTTGCTGTTAGCGGGGCTCCGCGGGGCTGCAAGACGCCCCACTAATTTGGCA
CAGGTAAAGCAAGTTGTACAAGGGAAAGAGGAAACGCCAGCCTCATTCTTAGAAAGATTA
AAAGAGGCTTACAGAATGTATACTCCCTATGACCCTGAGGACCCAGGGCAGGCTGCTAGT
GTTATCCTGTCCTTTATCTACCAGTCTAGCCCGGACATAAGAAATAAGTTACAAAGGCTA
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AAAAGGGAAACCCCAGAGGAAAGGGAAGAAAGATTATGGCAGCGGCAGGAAGAAAGAGAT
AAAAAGCGCCATAAGGAGATGACTAAAGTTCTGGCCACAGTAGTTGCTCAGAATAGAGAT
AAGGATAGAGGGAAAGTAAACTGGGAGATCAAAGGAAAATACCTCTGGGGAAAGACCAG
TGTGCCTATTGCAAGGAAAAGGGACATTGGGTTCGCGATTGCCCGAAACGACCCCGGAAG
AAACCCGCCAACTCCACTCTCCTCTAA

SEQ ID NO: 3 protein sequence of wild-type "env"

MANPSPPQIYNVTWVITNVQTNTQANATSMLGTLTDAYPTLHVDLCDLVGDTWEPIVLNP
TNVKHGARYSSSKYGCKTTDRKKQQQTYPFYVCPGHAPSLGPKGTHCGGAQDGFCAAWGC
ETTGEAWWKPTSSWDYITVKRGSSQDNSCEGKCNPLVLQFTQKGRQASWDGPKMWGLRLY
RTGYDPIALFTVSRQVSTITPPQAMGPNLVLPDQKPPSRQSQTGSKVATQRPQTNESAPR
SVAPTTMGPKRIGTGDRLINLVQGTYLALNATDPNKTKDCWLCLVSRPPYYEGIAILGNY
SNQTNPPPSCLSTPQHKLTISEVSGQGMCIGTVPKTHQALCNKTQQGHTGAHYLAAPNGT
YWACNTGLTPCISMAVLNWTSDFCVLIELWPRVTYHQPEYVYTHFAKAVRFRREPISLTV
ALMLGGLTVGGIAAGVGTGTKALLETA

SEQ ID NO: 4 protein sequence of wild-type "gag"

MGQTITTPLSLTLNHWSEVQARARNQGVEVRKKKWITLCEAEWVMMNVGWPREGTFTIDN
ISQVEERIFAPGPYGHPDQIPYITTWRSLATDPPPWVRPFLPPPKHPRTDPPEPLSPQPL
APQPSSPHPVLYPVLPKPDPPKAPVLPPNPSSPLIDLLTEEPPPYPGGHGPTPPSGPRTP
TASPIAIRLRERRENPAEKSQALPLREDPNNRPQYWPFSASDLYNWKLHNPPFSQDPVAL
TNLIESILVTHQPTWDDCQQLLQALLTAEERQRVLLEARKQVPGEDGRPTQLPNVVDEAF
PLTRPNWDFCTPAGREHLRLYRQLLLAGLRGAARRPTNLAQVKQVVQGKEETPASFLERL
KEAYRMYTPYDPEDPGQAASVILSFIYQSSPDIRNKLQRLEGLQGFTLSDLLKEAEKIYN
KRETPEEREERLWQRQEERDKKRHKEMTKVLATVVAQNRDKDRGESKLGDQRKIPLGKDQ
CAYCKEKGHWVRDCPKRPRKKPANSTLL

SEQ ID NO: 5 DNA sequence of FeLVgag

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GCCAGGGCCAGGAACCAGGGCGTGGAGGTGAGGAAGAAGAAGTGGATCACCCTGTGCGAG
GCCGAGTGGGTGATGATGAACGTGGGCTGGCCCAGGGAGGGCACCTTCACCATCGACAAC
ATCAGCCAGGTGGAGGAGAGGATCTTCGCCCCCGGCCCCTACGGCCACCCCGACCAGATC
CCCTACATCACCACCTGGAGGAGCCTGGCCACCGACCCCCCCCCCTGGGTGAGGCCCTTC
CTGCCCCCCCCCAAGCACCCCAGGACCGACCCCCCGAGCCCCTGAGCCCCAGCCCCTG
GCCCCCAGCCCAGCGCCCCCCCATGAGCAGCCTGTACCCCGTGCTGCCCAAGCCCGAC
CCCCCCAAGGCCCCCGTGCTGCCCCCCAACCCCAGCAGCCCCCTGATCGACCTGCTGACC
GAGGAGCCCCCCCCCTACCCCGGCGGCCACGGCCCCACCCCCCCCAGCGGCCCCAGGACC
CCCACCGCCAGCCCCATCGCCAGCAGGCTGAGGGAGAGGAGGGAGAACCCCGCCGAGAAG
AGCCAGGCCCTGCCCCTGAGGGAGGACCCCAACAACAGGCCCCAGTACTGGCCCTTCAGC
GCCAGCGACCTGTACAACTGGAAGCTGCACAACCCCCCCTTCAGCCAGGACCCCGTGGCC
CTGACCAACCTGATCGAGAGCATCCTGGTGACCCACCAGCCCACCTGGGACGACTGCCAG
CAGCTGCTGCAGGCCCTGCTGACCGCCGAGGAGAGGCAGAGGGTGCTGCTGGAGGCCAGG
AAGCAGGTGCCCGGCGAGGACGGCAGGCCCACCCAGCTGCCCAACGTGGTGGACGAGGCC
TTCCCCCTGACCAGGCCCAACTGGGACTTCTGCACCCCCGCCGGCAGGGAGCACCTGAGG
CTGTACAGGCAGCTGCTGCTGGCCGGCCTGAGGGGCGCCGCCAGGAGGCCCACCAACCTG
GCCCAGGTGAAGCAGGTGGTGCAGGGCAAGGAGGAGACACCCGCCAGCTTCCTGGAGAGG
CTGAAGGAGGCCTACAGGATGTACACCCCCTACGACCCCGAGGACCCCGGCCAGGCCACC
AGCGTGATCCTGAGCTTCATCTACCAGAGCAGCCCCGACATCAGGAACAAGCTGCAGAGG
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AACAAGAGGGAGACACCCGAGGAGAGGGAGGAGAGGCTGTGGCAGAGGCAGGAGGAGAGG
GACAAGAAGAGGCACAAGGAGATGACCAAGGTGCTGGCCACCGTGGTGGCCCAGAACAGG
GACAAGGACAGGGGCGAGAGCAAGCTGGGCGACCAGAGGAAGATCCCCCTGGGCAAGGAC
CAGTGCGCCTACTGCAAGGAGAAGGGCCACTGGGTGAGGGACTGCCCCAAGAGGCCCAGG
AAGAAGCCCGCCAACAGCACCCTGCTGTAG

SEQ ID NO: 6 FeLVgag protein sequence

MGQTITTPLSLTLNHWSEVQARARNQGVEVRKKKWITLCEAEWVMMNVGWPREGTFTIDN
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APQPSAPPISSLYPVLPKPDPPKAPVLPPNPSSPLIDLLTEEPPPYPGGHGPTPPSGPRT
PTASPIASRLRERRENPAEKSQALPLREDPNNRPQYWPFSASDLYNWKLHNPPFSQDPVA
LTNLIESILVTHQPTWDDCQQLLQALLTAEERQRVLLEARKQVPGEDGRPTQLPNVVDEA
FPLTRPNWDFCTPAGREHLRLYRQLLLAGLRGAARRPTNLAQVKQVVQGKEETPASFLER
LKEAYRMYTPYDPEDPGQATSVILSFIYQSSPDIRNKLQRLEGLQGFTLSDLLKEAEKIY
NKRETPEEREERLWQRQEERDKKRHKEMTKVLATVVAQNRDKDRGESKLGDQRKIPLGKD
QCAYCKEKGHWVRDCPKRPRKKPANSTLL

SEQ ID NO: 7 DNA sequence of FeLVenvgp85(-splice)

ATGGAGTCCCCCACCCACCCCAAGCCCTCCAAGGACAAGACCCTGTCCTGGAACATGGTG
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ATCTACAATGTGACCTGGGTGATCACCAATGTGCAGACCAACACCCAGGCCAATGCCACC
TCTATGCTGGGCACCCTGACAGATGCATACCCACCCTGCATGTGGACCTGTGTGACCTG
GTGGGGGACACCTGGGAGCCCATTCCGCTGAACCCCACCAATGTGAAGCATGGGGCCAGG
TACTCCTCCTCCAAGTATGGCTGCAAGACCACAGACAGGAAGAAGCAGCAGCAGACCTAC
CCCTTCTATGTGTGCCCTGGCCATGCCCCCTCCCTGGGCCCCAAGGGCACCCACTGTGGG
GGGGCCCAGGATGGCTTCTGTGCTGCCTGGGGCTGTGAAACCACAGGGGAGGCCTGGTGG
AAGCCCACCTCCTCCTGGGACTACATCACAGTGAAGAGGGGCTCCTCCCAGGACAACTCC
TGTGAGGGCAAGTGCAACCCCCTGGTGCTGCAGTTCACCCAGAAGGGCAGGCAGGCCTCC
TGGGATGGCCCCAAGATGTGGGGCCTGAGGCTGTACAGGACAGGCTATGACCCCATTGCC
CTGTTCACAGTGTCCAGGCAGGTGTCCACCATCACCCCCCCCCAGGCCATGGGCCCCAAC
CTGGTGCTGCCTGACCAGAAGCCCCCCTCCAGGCAGTCCCAGACAGGCTCCAAGGTGGCC
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GTGAGGTTCAGGAGGGAGCCCATCTCCCTGACAGTGGCCCTGATGCTGGGGGGCCTGACA
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CCCTGGCTTACAACCCTAATTTCGTCTATTATGGGCCCCTTGCTTATCCTGCTCCTAATT
CTCCTCTTCGGCCCATGCATCCTTAACCGATTGGTGCAATTCGTAAAAGACAGAATATCG
GTGGTACAAGCCTTAGTTTTAACCCAACAGTACCAACAGATAAAGCAATACGATCCGGAC
CGACCATGA

SEQ ID NO: 8 DNA sequence of FeLVenvgp70(-splice)

ATGGAGTCCCCCACCCACCCCAAGCCCTCCAAGGACAAGACCCTGTCCTGGAACATGGTG
TTCCTGGTGGGCATCCTGTTCACCATTGACATTGGCATGGCCAACCCCTCCCCCCCCGG
ATCTACAATGTGACCTGGGTGATCACCAATGTGCAGACCAACACCCAGGCCAATGCCACC
TCTATGCTGGGCACCCTGACAGATGCATACCCCACCCTGCATGTGGACCTGTGTGACCTG
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TACTCCTCCTCCAAGTATGGCTGCAAGACCACAGACAGGAAGAAGCAGCAGCAGACCTAC
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ACCCAGAGGCCCCAGACCAATGAGTCTGCCCCCAGGTCTGTGGCCCCCACCACCATGGGC
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CCCTACTATGAGGGCATTGCCATCCTGGGCAACTACTCCAACCAGACCAACCCCCCCCCC
TCCTGCCTGTCCACCCCCCAGCACAAGCTGACCATCTCTGAGGTGTCTGGCCAGGGCATG
TGCATTGGCACAGTGCCCAAGACCCACCAGGCCCTGTGCAACAAGACCCAGCAGGGCCAC
ACAGGGGCCCACTACCTGGCTGTCCCCAATGGCACCTACTGGGCCTGCAACACAGGCCTG
ACCCCCTGCATCTCCATGGCTGTGCTGAACTGGACCTCTGACTTCTGTGTGCTGATTGAG
CTGTGGCCCAGGGTGACCTACCACCAGCCTGAGTATGTGTACACCCACTTTGCCAAGGCT
GTGAGGTTCAGGAGGGAGCCCATCTCCCTGACAGTGGCCCTGATGCTGGGGGGCCTGACA
GTGGGGGGCATTGCTGCTGGGGTGGGCACAGGCACCAAGGCCCTGCTGGAAACAGCCTGA

SEQ ID NO: 9 protein sequence of FeLVenvgp85(-splice)

MESPTHPKPSKDKTLSWNMVFLVGILFTIDIGMNPSPPRIYNVTWVITNVQTNTQANAT
SMLGTLTDAYPTLHVDLCDLVGDTWEPIPLNPTNVKHGARYSSSKYGCKTTDRKKQQQTY
PFYVCPGHAPSLGPKGTHCGGAQDGFCAAWGCETTGEAWWKPTSSWDYITVKRGSSQDNS
CEGKCNPLVLQFTQKGRQASWDGPKMWGLRLYRTGYDPIALFTVSRQVSTITPPQAMGPN
LVLPDQKPPSRQSQTGSKVATQRPQTNESAPRSVAPTTMGPKRIGTGDRLINLVQGTYLA
LNATDPNKTKDCWLCLVSRPPYYEGIAILGNYSNQTNPPPSCLSTPQHKLTISEVSGQGM
CIGTVPKTHQALCNKTQQGHTGAHYLAVPNGTYWACNTGLTPCISMAVLNWTSDFCVLIE
LWPRVTYHQPEYVYTHFAKAVRFRREPISLTVALMLGGLTVGGIAAGVGTGTKALLETAQ
FRQLQMAMHTDIQALEESVSALEKSLTSLSEVVLQNRRGLDILFLQEGGLCAALKEECCF
YADHTGLVRDNMAKLRERLKQRQQLFDSQQGWFEGWFNKSPWLTTLISSIMGPLLILLLI
LLFGPCILNRLVQFVKDRISVVQALVLTQQYQQIKQYDPDRP

SEQ ID NO: 10 protein sequence of FeLVenvgp70(-splice)

MESPTHPKPSKDKTLSWNMVFLVGILFTIDIGMANPSPPRIYNVTWVITNVQTNTQANAT
SMLGTLTDAYPTLHVDLCDLVGDTWEPIPLNPTNVKHGARYSSSKYGCKTTDRKKQQQTY
PFYVCPGHAPSLGPKGTHCGGAQDGFCAAWGCETTGEAWWKPTSSWDYITVKRGSSQDNS
CEGKCNPLVLQFTQKGRQASWDGPKMWGLRLYRTGYDPIALFTVSRQVSTITPPQAMGPN
LVLPDQKPPSRQSQTGSKVATQRPQTNESAPRSVAPTTMGPKRIGTGDRLINLVQGTYLA
LNATDPNKTKDCWLCLVSRPPYYEGIAILGNYSNQTNPPPSCLSTPQHKLTISEVSGQGM
CIGTVPKTHQALCNKTQQGHTGAHYLAVPNGTYWACNTGLTPCISMAVLNWTSDFCVLIE
LWPRVTYHQPEYVYTHFAKAVRFRREPISLTVALMLGGLTVGGIAAGVGTGTKALLETA

Fig. 1 Comparison of *in vitro* expression of wt and synFeLVgag sequence of the FeL virus

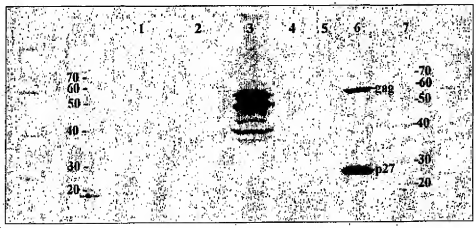

Fig. 2 Comparison of *in vitro* expression of wt and FeLVenvp85 sequence of the FeL virus

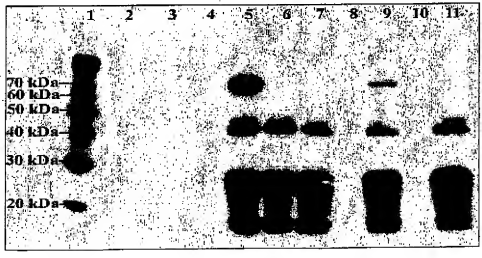

Fig. 3: *In vivo* expression of the "env" protein